EXHIBIT C

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Experimental therapy of systemic lupus erythematosus: the treatment of NZB/W mice with mouse soluble interferon-γ receptor inhibits the onset of glomerulonephritis*

Female NZB/W F1 mice develop an autoimmune disease similar to human systemic lupus erythematosus (SLE), and ultimately die of glomerulonephritis. Starting at the age of 16 weeks NZB/W F1 mice were treated for a period of 19 weeks with soluble interferon-γ receptor (sIFN-γR), anti-IFN-γ monoclonal antibody (mAb) or IFN-γ. All mice treated with sIFN-γR or anti-IFN-γ mAb were alive 4 weeks after the treatment was discontinued, whereas 50% of mice died in the placebo groups and 78% of the mice died in the IFN-γ-treated group. Histologically, there was severe membrano-proliferative glomerulonephritis in IFN-γ- and placebo-treated mice, and minimal or no mesangioproliferative disease in mice receiving sIFN-γR or anti-IFN-γ mAb. The renal mononuclear infiltrate (T lymphocytes and monocytes), expression of major histocompatibility complex class II antigen and glomerular immunoglobulin and complement deposition were reduced in those mice. These data suggest that an IFN-γ inhibitor, such as the soluble IFN-γR, can be used for SLE therapy in the early stages of the disease.

1 Introduction

The (NZBxNZW)F1 mice (NZB/W) develop an autoimmune disease similar to human SLE. Female mice die from glomerulonephritis during the first year of life whereas males develop the disease during the second year of life [1]. In this mouse model of lupus, cytokines play a pathogenic role that is not clearly understood. The onset of the disease is accelerated by the administration of IFN-γ or TNF-α and delayed by a chronic treatment with a neutralizing anti-IFN-γ mAb [2, 3]. In other experiments, TNF-α or anti-IL-10 mAb delayed the development of the disease in NZB/W female mice [4–6] and neutralizing anti-TNF-α antibodies reversed the protective effect of anti-IL-10 antibodies [6]. Taking in account these paradoxical results, the SLE-like disease expressed in the NZB/W female mice should be mediated by cytokines released by both Th1 and Th2 cells.

In humans, the manifestations of SLE include arthritis, skin rashes, and glomerulonephritis, while autoimmune thrombocytopenia, hemolytic anemia, pericarditis and pathology of the central nervous system are common complications [7]. Human SLE is also characterized by an abnormal expression of cytokines. SLE-derived peripheral blood mononuclear cells stimulated *in vitro* with mitogens produce decreased amounts of IL-2, IFN-γ and TNF-α. On the other hand, unstimulated peripheral blood mononuclear cells with increased levels of IL-2, IFN-γ and TNF-α mRNA and high titers of serum IL-2, IFN-γ and TNF-α are found in patients with active SLE. Moreover, the presence of IL-1, IL-6 and IFN-γ in nephritic kidneys suggests that they have local pathogenic effects (reviewed in [8]). Altogether these data indicate that active human SLE is mediated by Th1 cells. This idea is further supported by the observation that patients with chronic myelogenous leukemia treated with combinations of IFN-α and IFN-γ developed a lupus-like autoimmune disease [9].

Our recent effort was focused on the study of IFN-γ, a Th1 cytokine, in the pathogenesis of SLE and on the evaluation of IFN-γ inhibitors in the therapy of this autoimmune disease. Since mAb cannot be used for chronic treatment of human diseases, we investigated the possibility to apply therapeutically an IFN-γ inhibitor such as a soluble form of the IFN-γR (sIFN-γR). With this aim, we constructed a soluble form of the mouse IFN-γR and assessed its capacity to antagonize mouse IFN-γ *in vivo* [10–12]. We report here that the treatment with sIFN-γR, is effective if initiated at 4 months of age, when NZB/W F1 females show circulating anti-double-stranded (ds)DNA antibodies but not proteinuria. In these conditions, the treatment with sIFN-γR blocks the infiltration of T lymphocytes and monocyte/macrophages, inhibits the epithelial expression of MHC class II antigens in the kidney and minimizes the autoimmune glomerulonephritis.

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Abbreviations: **NZB/W:** New Zealand Black x New Zealand White F1 mice **sIFN-γR:** Soluble IFN-γ receptor

Key words: Soluble interferon-γ receptor / Glomerulonephritis / NZB/W mice

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2 Materials and methods

2.1 Mice

NZB/W (New Zealand Black × New Zealand White) females, 2 months old, were purchased from Bomholtgard (Denmark) and maintained in our animal facilities under specific pathogen-free (SPF) conditions. Animals were randomly distributed in the different treatment groups.

2.2 Mouse IFN-γ and anti-IFN-γ mAb

Recombinant IFN-γ was expressed in *E. coli* [13] and purified according to Doebeli et al. [14]. The pure IFN-γ displays a specific antiviral activity of 10^7 IU/mg of protein.

XMG1,2 is a rat mAb neutralizing the activity of IFN-γ [15].

2.3 Mouse sIFN-γR

The recombinant sIFN-γR was expressed in Sf9 insect cells infected with a baculovirus transfer vector carrying the cDNA encoding the extracellular domain of the cellular receptor [11]. The purified sIFN-γR is a 32 kDa glycoprotein that binds IFN-γ with an estimated Kd of 0.1–0.3 nM. *In vitro,* it competes with the cellular receptor for the binding of IFN-γ ($IC_{50} = 0.72$ nM) and inhibits the antiviral activity exerted by IFN-γ. After injection in mice, the sIFN-γR shows a blood persistence of 1 h, is not immunogenic and specifically inhibits IFN-γ [10, 12].

2.4 Treatment of NZB/W mice with sIFN-γR or IFN-γ

Mice were injected ip with 100 μg sIFN-γR three times per week. Considering the 1 h blood persistence of the sIFN-γR, as well as its capacity to antagonize the antiviral activity of IFN-γ on L929 mouse fibroblasts ($IC_{50} = 15$ nM) [12], we calculated that injections of 100 μg sIFN-γR three times a week should be sufficient to maintain a concentration in the blood which is able to neutralize 10 U/ml of IFN-γ, a concentration typically detected in body fluids [16]. A second group received 5×10^4 U IFN-γ three times per week, according to Jacob et al. [2]. The treatment control group was injected i.p. with 100 μg anti-IFN-γ mAb XMG1,2 once a week. The mAb XMG1,2 inhibits the antiviral activity of IFN-γ with an IC_{50} of 0.1 nM and can be traced in the blood for at least 72 h (unpublished results). Thus, one weekly injection of 100 μg XMG1,2 is sufficient to maintain a blood concentration able to neutralize the endogenous IFN-γ. The placebo groups received either the carrier solution (saline) or 100 μg irrelevant rat IgG (Sigma, St. Louis, MO) once a week i.p.

Two treatment schedules were chosen. In the first protocol, 4-month-old NZB/W females (nine per group) with blood anti-dsDNA antibodies but not proteinuria, were treated for a period of 19 weeks. The second protocol consisted in a late treatment initiated at the age of 7 months in groups of seven mice for a period of 12 weeks. The 7-months-old NZB/W females are characterized by high levels of circulating anti-dsDNA antibodies and starting proteinuria.

2.5 Detection of serum IgG levels and anti-dsDNA autoantibodies

Serum IgG and anti-dsDNA autoantibodies were quantified by ELISA every month. Total IgG were measured on microtiter plates coated with goat anti-mouse IgG (Jackson, West Grave, PA) using a goat anti-mouse IgG coupled to alkaline-phosphatase (Cappel, West Chester, PA) as second reagent. Sera were diluted from 10^{-5} to 10^{-6} and the IgG concentration was calculated with reference to an internal standard of pure mouse IgG (Sigma). IgG anti-dsDNA autoantibodies were measured on microtiter plates coated with dsDNA (Sigma) at 10 μg/ml PBS [17]. The mouse sera were incubated at a 1/500 dilution and IgG specific for anti-dsDNA were detected using an alkaline phosphatase-linked goat anti-mouse IgG (Cappel). The results are expressed in absorbance units measured at 405 nm (A_{405}). In each test, sera from an adult C57BL/6 lpr/lpr and (C57BL/6 x DBA/2) F1 mice were included as positive and negative controls, respectively.

2.6 Assessment of renal disease

Renal disease was assessed by the development of proteinuria and by histological evaluation of the kidney. Proteinuria was measured colorimetrically using commercially available sticks (Boehringer, Mannheim, FRG) and graded according the following code: 0, <0.3 mg/ml; 1+, 0.3 mg/ml; 2+, 1 mg/ml; 3+, 5 mg/ml. Protein concentrations over mg/ml urine were considered positive. Kidney histology was performed on mice that died or were killed during the treatment, or on mice that were killed 2 months after the end of the treatment. The kidneys were removed, fixed in 4% phosphate-buffered formaldehyde and processed for histological examination. Sections of 5 μm were stained with hematoxylin-eosin, chromotrope anilin blue [18]. Kidneys were analyzed for mesangio-proliferative and membrano-proliferative glomerulonephritis with capsular adhesions and fibrosis [19]. The severity of the changes was assessed by a semi-quantitative score (0: none, 1: minimal, 2: slight, 3: moderate, 4: distinct, 5: severe). The percentage of glomeruli with protein, *e.g.* complement and Ig deposits was evaluated by the observation of 25 glomeruli. For immunochemistry, renal tissue cryostat sections of 5 μm were fixed in acetone, air dried and stored at − 70 °C until use. Slides were incubated with rat mAb against mouse IgG and complement factor C3 (Nordic, Tilburg, The Netherlands) in order to characterize the protein deposits. The infiltrating cells were stained with rat mAb: anti-mouse monocytes/macrophages (F4/80), anti-CD3 (KT3 1.1), and anti-class II (M5/114) obtained from Prof. Hans Hengartner, University of Zurich. A goat anti-rat Ig coupled with phosphatase (Tago, Glostrup, Denmark) was used as secondary antibody [20].

2.7 Detection of anti-IFN-γR antibodies

Antibodies against the IFN-γR were assayed on sIFN-γR coated microtiter plates as previously described [12].

2.8 Statistical analysis

Differences in serological parameters between the groups were evaluated with Mann-Whitney's rank-sum test. A p value < 0.05 was considered significant.

3 Results

3.1 Characteristics of NZB/W female mice used in the experiments

At the age of 4 months, when the treatment was initiated, the NZB/W females showed serum levels of 5.4 mg/ml total IgG (range 3.1–11 mg/ml) and 0.21 A_{405} units of IgG anti-dsDNA (range 0.10–0.64 A_{405} units). At this age, the animals did not show proteinuria. At the age of 7 months, when the late treatment schedule was initiated, serum levels of 9.3 mg/ml IgG (range 2.3–24 mg/ml) and 1.17 A_{405} units of IgG anti-dsDNA (range 0.08–3.00 A_{405} units) were measured. At this age, NZB/W females develop proteinuria. Serum levels of about 4 mg/ml IgG and anti-dsDNA autoantibodies of less than 0.10 A_{405} units were detected in 4–8-month-old females from normal mouse strains. Non-autoimmune mice never showed proteinuria.

3.2 Effects of treatment with sIFN-γR, anti-IFN-γ mAb or IFN-γ on mortality

Fig. 1 shows the mortality of the treated mice. In groups that received placebo, 50% of the animals died before the age of 38 weeks. The treatment with IFN-γ increased mortality, as 50% of the animals were dead after 10 weeks of treatment, *i.e.* at the age of 30 weeks. In addition, this treatment induced ascites and anasacra. By contrast, all *mice treated with either sIFN-γR or anti-IFN-γ mAb* were alive at the age of 40 weeks. At the end of the experiment, when the NZB/W mice were 45 weeks old and the treatment had been discontinued for 10 weeks, all the mice treated with IFN-γ and 71% of the mice treated with placebo were dead, in comparison to 23% and 17% in the groups treated with sIFN-γR or anti-IFNγ mAb, respectively.

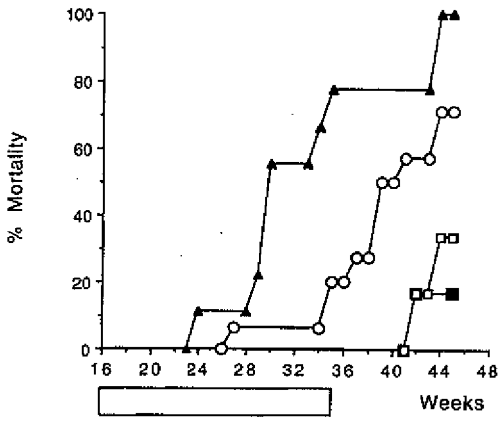

Figure 1. Mortality of the treated NZB/W mice. Mice were injected from the age of 4 months for a period of 19 weeks as indicated by the rectangle under the figure. Mice were treated with 100 µg sIFN-γR three times/week (□), with 100 µg anti-IFN-γ mAb once a week (■), or with 5 × 10^4 U IFN-γ three times/week (▲) and compared with placebo (○).

The mice treated with either of the two placebos (saline or irrelevant rat IgG) gave superimposable results and were always considered as a single group.

3.3 Effects of treatment with sIFN-γR, anti-IFN-γ mAb or IFN-γ on proteinuria

The progression of proteinuria is shown in Table 1. In the placebo group, mild proteinuria (1 mg/ml) was detected at the age of 31 weeks and proteinuria of 5 mg/ml or above were reached 2 weeks later. The animals treated with IFN-γ showed mild proteinuria already after 7 weeks of treatment at the age of 23 weeks and reached the maximum 2 weeks later. Treatment with sIFN-γR or anti-IFN-γ mAb significantly delayed the development of proteinuria, which reached the maximum (5 mg/ml) after 19 weeks of treatment at the age of 37 weeks. Mild proteinuria was detected in mice treated with sIFN-γR after 15 weeks of treatment (age of 33 weeks).

Table 1. Development of proteinuria in NZB/W mice during the treatment with sIFN-γR or IFN-γ

Age (weeks)	Treatment	Proteinuria (mg/ml) in NZB/W mice treated with			
		placebo	IFN-γ	mAb to IFN-γ	sIFN-γR
16	Start	<0.30	<0.30	<0.30	<0.30
23		<0.30	1	<0.30	<0.30
31		1	5	<0.30	<0.30
35	End	5	5	<0.30	1
37		5	5	5	5

Proteinuria was measured in pools of urine of four to five mice.

3.4 Effects of treatment with sIFN-γR, anti-IFN-γ mAb or IFN-γ on serum IgG levels

Table 2 shows the serum IgG concentrations in NZB/W mice during and after the discontinuation of the treatment. Before the treatment, at 16 weeks of age, the serum IgG level ranged between 3.2 and 11 mg/ml, with a mean ± SEM value of 5.5 ± 0.36 mg/ml. Mice treated with placebo showed a steadily increment of IgG serum concentration through all the period of observation. Although the differences were statistically not significant ($p > 0.05$), the

Table 2. IgG levels in NZB/W mice in the different treatment groups[a)]

Age (weeks)	IgG levels (mg/ml)			
	placebo	IFN-γ	mAb to IFN-γ	sIFN-γR
16	5.5 ± 0.8	5.7 ± 0.7	6.1 ± 0.9	5.4 ± 0.6
27	8.8 ± 2.0	3.9 ± 1.3	4.8 ± 1.0	5.6 ± 0.8
33	11.3 ± 3.3	6.8 ± 2.8	7.4 ± 1.6	5.9 ± 1.2
40	15.5 ± 6.1	6.7[b)]	10.6 ± 4.3	7.8 ± 2.0

a) Mice were treated from 16 to 35 weeks of age. Results are expressed as means ± SEM.

b) All the mice died but one.

IgG levels measured during the treatment period, were appreciably lower in mice injected with either sIFN-lγR or anti-IFN-γ than in the placebo group. When the treatment with IFN-γ antagonists was discontinued (35 weeks of age) serum IgG levels increased in both groups. Most of the IFN-γ-treated mice died without any significant increase of IgG levels. At the age of 27 weeks, six out of nine mice had developed hypogammaglobulinemia with IgG levels below 2.5 mg/ml.

3.5 Effects of treatment with sIFN-γR, anti-IFN-γ mAb or IFN-γ on anti-dsDNA

Table 3 shows the serum levels of IgG anti-dsDNA during and after the treatment. Before the treatment, the serum levels ranged between 0.10 and 0.64 A_{405} units with a mean (± SEM) value of 0.21 ± 0.01. Under the same conditions, negative control serum (C57BL/6 × DBA/2) F1 and positive control serum (C57BL/6 lpr/lpr) showed anti-dsDNA levels of 0.05 and 2.45 A_{405} units, respectively. No statistically significant difference between the different groups was observed ($p > 0.05$) and autoantibodies levels steadily increased in the different treatment groups. All the placebo-treated mice (15/16) had anti-dsDNA levels higher than 0.5 A_{405} unit at the age of 33 weeks, whereas only four out of nine and six out of nine mice treated with anti-IFN-γ mAb and sIFN-γR, respectively, reached the same level.

Table 3. IgG anti-dsDNA autoantibody levels in NZB/W mice in the different treatment groups[a]

Age (weeks)	IgG anti-dsDNA autoantibody levels (A units)			
	placebo	IFN-γ	mAb to IFN-γ	sIFN-γR
16	0.21 ± 0.03	0.20 ± 0.03	0.21 ± 0.05	0.18 ± 0.01
27	0.99 ± 0.29	0.67 ± 0.14	0.60 ± 0.29	0.30 ± 0.06
33	1.48 ± 0.35	0.90 ± 0.21	0.97 ± 0.34	1.03 ± 0.25
40	2.43 ± 0.47	2.09[b]	1.97 ± 0.41	1.76 ± 0.33

[a] Mice were treated from 16 to 35 weeks of age. Results are expressed as means ± SEM.

[b] All the mice died but one.

3.6 Effects of treatment with sIFN-γR, anti-IFN-γ mAb or IFN-γ on the progression of glomerulonephritis

Histology of the kidneys was first performed on mice that died or were killed during the treatment period. In the placebo group, most of the mice had advanced mesangio-proliferative glomerulonephritis (Table 4) with enlargement of the glomeruli (Fig. 2 A), mesangial cell proliferation, Ig and complement deposits, fibrosis, capillary lumen obliteration and casular adhesion. Immunohistology showed a significant infiltration of T-lymphocytes (CD3^{+} cells) and macrophages (F4/80^{+} cells). The infiltration was mainly confined to the periglomerular area and the adjacent interstitium (Table 4). MHC class II antigens were significantly expressed by the glomerulus and tubular epithelium (Table 4). The mice treated with anti-IFN-γ mAb or sIFN-γR, presented only minimal signs of mesangio-proliferative glomerulonephritis with normal size glomeruli, "patent" capillaries (Table 4, Fig. 2 C and D). Immunohistology showed minimal kidney infiltration of T lymphocytes and macrophages, low expression of MHC class II antigens and reduced deposition of Ig and complement (Table 4). The IFN-γ-treated mice developed the most severe form of membrano-proliferative glomerulonephritis (Table 4, Fig. 2 B) with a vast T-lymphocyte and macrophage infiltration, high expression of MHC class II antigens and deposition of Ig and complement (Table 4). Histology performed 2 months after the end of the treatment confirmed the long-term beneficial effect of anti-IFN-γ mAb or sIFN-γR treatment (Table 5). Early treatment of mice with anti-IFN-γ mAb or sIFN-γR resulted in a lesser degree of membrano-proliferative glomerulonephritis, while the IFN-γ-treated mice had the most severe form and died.

3.7 Late treatment

In a second protocol, NZB/W females were treated with saline, anti-IFN-γ mAb, or sIFN-γR starting at the age of 7 months. At that time, the mean IgG levels were 9.3 mg/ml, and a mean value of 1.17 A_{405} units was measured for anti-dsDNA autoantibodies and the proteinuria was starting. Under these conditions, the treatment with sIFN-γR or anti-IFN-γ mAb did not change the

Table 4. Renal histology in NZB/W mice that died or that were killed during the early treatment time period[a]

Glomerulonephritis		NZB/W mice treated with			
		Placebo	mAb to IFN-γ	sIFN-γR	IFN-γ
Mesangio-proliferative		3.4 ± 0.7	0.7 ± 0.3	2.0 ± 1.0	3.8 ± 0.2
Membrano-proliferative		3.8 ± 0.6	0.3 ± 0.3	1.3 ± 1.3	4.2 ± 0.4
Periglobular infiltrate		2.6 ± 0.5	0.7 ± 0.3	0.7 ± 0.3	3.4 ± 0.2
Tubular regeneration		3.2 ± 0.6	0	1.3 ± 1.3	3.2 ± 0.4
Protein deposits in glomeruli		3.0 ± 0.8	0.3 ± 0.3	0.3 ± 0.3	3.8 ± 0.2
MHC class II expression	Tubular epithelium	3.2 ± 0.2	1.0 ± 0.6	1.7 ± 0.7	5.0 ± 0.0
	Glomerulus	2.2 ± 0.2	0.3 ± 0.3	2.3 ± 0.9	3.7 ± 0.3
Cell infiltration	T cells (CD3^{+})	2.5 ± 0.3	0.7 ± 0.3	1.3 ± 0.3	5.0 ± 0.0
	Macrophages (F4/80^{+})	3.3 ± 0.2	1.0 ± 0.0	1.3 ± 0.3	3.7 ± 0.3

[a] Results are expressed as mean ± SEM of semi-quantitative score (0–5). In the placebo and IFN-γ groups, the histology was performed on five mice while in the other groups on three mice. In the placebo group, the immunohistology was performed on four mice while in the other groups on three mice.

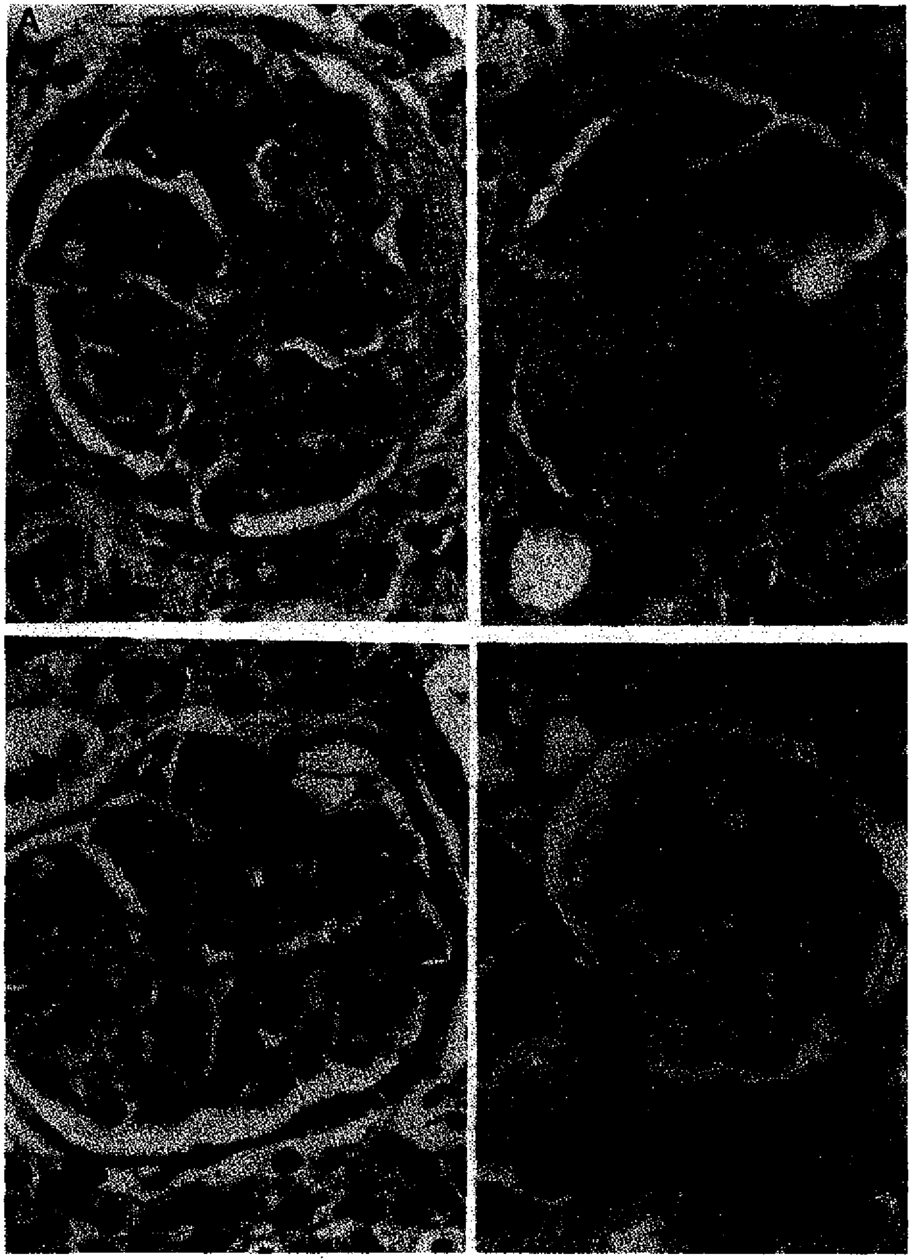

Figure 2. Glomerular changes in 35-week-old NZB/W mice after 19 weeks of treatment. (A) Placebo: Severe membrano-proliferative glomerulonephritis with proliferation of the mesangium, epithelium, obliteration of the capillary lumen and capsular adhesion. (B) IFN-γ: Severe membrano-proiferative glomerulonephritis with protein deposits comparable to A. (C) anti-IFN-γ mAb: Normal sized glomeruli with patent capillary lumens and minimal mesangio-proliferation. (D) sIFN-γR: Minimal mesangio-proliferation.

Table 5. Renal histology in NZB/W mice killed 2 months after the discontinuation of the treatment in the early treatment experiment

Glomerulonephritis			NZB/W mice treated with		
		Placebo	mAb to IFN-γ	sIFN-γR	IFN-γ
Mesangio-proliferative		2.8 ± 0.2	1.8 ± 0.4	1.4 ± 0.7	5.0
Membrano-proliferative		4.3 ± 0.2	3.2 ± 0.6	2.2 ± 0.5	5.0
Periglobular infiltrate		2.7 ± 0.2	2.8 ± 0.5	1.8 ± 0.2	4.0
Tubular regeneration		3.3 ± 0.6	1.4 ± 0.7	2.2 ± 1.0	4.0
Protein deposits in glomeruli		3.0 ± 0.4	1.6 ± 0.4	1.2 ± 0.6	5.0
MHC class II expression	Tubular epithelium	3.0 ± 0.3	2.0 ± 0.6	2.0 ± 0.4	3.0
	Glomerulus	3.33 ± 0.2	2.6 ± 0.2	2.0 ± 0.3	4.0
Cell infiltration	T cells [CD3⁺]	2.0 ± 0.0	1.8 ± 0.2	1.4 ± 0.2	4.0
	Macrophages [F4/80⁺]	3.17 ± 0.2	2.6 ± 0.4	2.2 ± 0.4	4.0

a) Results are expressed as mean ± SEM of semi-quantitative score (0–5). In the placebo group, the histology and the immunohistology were performed on six mice, in the mAb and sIFN-γR on five mice and IFN-γ group on one mouse.

survival rate: 50% mortality was reached at 48, 48 and 42 weeks of age in the placebo, anti-IFN-γ mAb, and sIFN-γR treatment groups, respectively. This late treatment influenced neither proteinuria nor the levels of IgG or anti-dsDNA autoantibodies and no differences were observed in histology (data not shown).

3.8 Anti-IFN-γR antibodies after sIFN-γR treatment

After 2 and 4 months of treatment with sIFN-γR (100 μg per mouse, three times a week), NZB/W mice did not develop significant titers (< 500) of antibodies against the receptor for IFN-γ. Upon identical treatment, BALB/c mice of the same age developed high titers (> 20 000) of antibodies when injected with human sIFN-γR and titers up to 3000 when injected with the sIFN-γR. The sera from NZB/W mice of the same age that were treated with placebo were negative.

4 Discussion

This report presents the results of a chronic treatment of NZB/W female mice with sIFN-γR at different stages of the disease expression. As already observed [12], this treatment did not induce toxic effects. When the treatment started in 7-month-old NZB/W females with high blood level of anti-dsDNA antibodies and starting proteinuria, the progression of the lupus-like disease was not affected. In these conditions, the control treatment with anti-IFN-γ mAb was similarly ineffective. When the treatment with sIFN-γR started in 4-month-old NZB/W females with auto-antibodies to dsDNA but no signs of proteinuria, the progression of the lupus-like disease was delayed or stopped. Histology showed that this improvement mainly concerned protection from glomerulonephritis, leading to increased survival. The treatment with sIFN-γR produced long-lasting effects, with minimal glomerulonephritis when compared to placebo 2 months after discontinuation of treatment. The serologic parameters (hypergammaglobulinemia and anti-dsDNA autoantibodies) did not show any significant inhibition but their onset was delayed. Proteinuria developed immediately after the discontinuation of the treatment. A similar picture was observed when the mice received the control treatment with rat anti-IFN-γ mAb.

The inhibition of the SLE-like disease without an actual reduction of autoantibody levels we observed with IFN-γ antagonists, was already described in studies on NZB/W mice treated with hydrocortisone, androgens, prostaglandin E2, total lymphoid irradiation or the immunosuppressant CP17193 [21]. Since the levels of autoantibodies rise before the kidney damage occurs, and anti-DNA IgG can be eluted from the kidneys [22], it is accepted that the SLE glomerulonephritis results from the deposition of immune complexes. However, the immune complexes accumulation in the glomeruli cannot simply be attributed to hypergammaglobulinemia and anti-DNA autoantibodies [23]. The dissociation between autoantibody levels and glomerulonephritis is further supported by the fact that most of the NZB/W mice treated with IFN-γ showed a clear acceleration of the disease with a significant decrease of IgG or anti-dsDNA antibodies. This is in line with the findings that IFN-γ is a potent inhibitor of antigen-specific B cell differentiation and gives only a modest enhancement of IgG2a production [24, 25]. The histological analysis of the kidneys from NZB/W mice treated with sIFN-γR shows less Ig and complement deposits than placebo-treated mice. Since IFN-γ increases the synthesis of C3 and C4 complement factors by glomerular mesangial cells [26], the depletion of endogenous IFN-γ may affect the local complement production and the clearance of immune complexes. Thus, the IFN-γ deprivation obtained by sIFN-γR could decrease the extent of glomerular protein deposition without affecting the circulating autoantibody levels. The treatment with sIFN-γR also drastically reduced the infiltration of T cells and macrophages and the expression of MHC class II antigens by the glomerulus and tubular epithelium in the kidneys of NZB/W mice.

Although, we never detected blood IFN-γ in NZB/W mice (data not shown), the role of Th1 and IFN-γ in promoting the pathogenesis of the severe membrano-proliferative glomerulonephritis is supported by several reports. IFN-γ was detected in the kidneys of SLE patients [8] and was described as the mediator that is continually required for tissue infiltration of Th1 lymphocytes and of vascular leakage [27, 28]. Moreover, IFN-γ may promote the kidney lesions through its multiple proinflammatory activities. IFN-γ may facilitate *"in loco"* presentation of antigen by inducing MHC class I and II antigen expression in macrophages, endothelial or epithelial cells and activate macrophages to an enhanced release of cytokines (IL-1 and TNF), active oxigen intermediates and NO^- [29].

Recently, Ishida et al. [6] reported that the treatment of NZB/W female mice with anti-IL-10 mAb from birth until 38–42 weeks of age delayed the onset of autoimmunity. Conversely, the administration of IL-10 from 4 to 38 weeks of age accelerated the development of the disease. The anti-SLE effect of IL-10 neutralizing antibodies was reversed by anti-TNF-α mAb. In a previous report [4], the treatment of 16-week-old NZB/W mice with TNF-α also inhibited the autoimmune nephritis. In contrast to the results obtained with IFN-γ antagonists in NZB/W mice ([2] and this report), these data define this SLE disease as Th2 dependent. Since several activities of IFN-γ and TNF-α are synergistic and IL-10 inhibits IFN-γ production and activity as well as cell-mediated immunity [30–32], the protective effects of TNF-α, anti-IL-10 mAb, anti-IFN-γ mAb or sIFN-γR are paradoxical. However, the treatment with IL-10 or anti-IL-10 mAb was applied to newborn mice while the treatments with sIFN-γR, IFN-γ or TNF-α were given to 16-weeks-old NZB/W mice. Such opposite effects according to the cytokine dosage and the age of animals at the initiation of the treatment was already described in autoimmune diseases ([33]; Boissier et al., manuscript in preparation). The treatment of 16-weeks-old NZB/W mice with 30 μg TNF-α per mouse per week inhibited glomerulonephritis [4]. In similar conditions, the treatment of NZB/W mice with lower dosages of TNF-α (0.6–6 μg per mouse per week) accelerated the disease [3].

Peritoneal macrophages from NZB/W mice show a decreased capacity to produce TNF-α [4] but since an enhanced steady state level of mRNA for TNF-α is detectable in the renal cortex [3], the peripheral impairment of TNF-α production cannot be attributed to a restriction fragment length polymorphism in the TNF-α gene [4]. The so called replacement therapy with high dosages of TNF-α may protect the mice by a down-regulation of the kidney production of TNF-α and expression of IFN-γ-induced MHC class II expression [34] while the injection of low dosages of TNF-α may be uneffective on the local production of TNF-α and accelerate the onset of nephritis. Since patients treated with combinations of IFN-α and IFN-γ developed a lupus-like autoimmune disease [9], and since IFN-γ and IFN-γ antagonists never gave paradoxical results when injected in NZB/W mice with autoantibodies and without proteinuria, we believe that SLE-like nephritis is a Th1-dependent autoimmune disease where IFN-γ plays an univocal pathogenic role. On the other hand, the development of autoantibodies is not affected by the administration or the depletion of IFN-γ and B cells from NZB/W mice are hyperresponsive to IL-5 [35]. It can be assumed a Th2 control for this aspect of the SLE-like disease.

The therapy of SLE is mainly palliative, based on anti-inflammatory corticosteroids and immunosuppressive agents [36] that is applied during the phases of active disease and clinical exacerbation [37]. The efficacy of the sIFN-γR treatment in NZB/W mice suggests a novel and not toxic therapeutic approach to human SLE. Provided that kidneys are not yet irreversibly damaged, the use of human soluble or chimeric receptors (constant part of human Ig heavy chain linked to the human soluble IFN-γ receptor) [38, 39] is an attractive new perspective in the treatment of autoimmune glomerulonephritis in human SLE.

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